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*EXTRANUCLEAR TRANSMISSION IN YEAST HETEROKARYONS**

BY ROBERT E. WRIGHT AND JOSHUA LEDERBERG†

DEPARTMENT OF GENETICS, UNIVERSITY OF WISCONSIN, MADISON, WISCONSIN

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Fowell[1] has given preliminary evidence for transient heterokaryosis, incidental to zygote formation, in a strain of *Saccharomyces cerevisiae* var. *ellipsoideus*. This paper constitutes (1) a verification of Fowell's scheme and (2) its application to the demonstration of cytoplasmic transfer of factors associated with respiratory deficiency.[2]

Stocks.—The cultures WY42 (α) and WY44 (a) were kindly supplied by R. R. Fowell as HMh^+ and $HQ10c^-$, respectively. They grew dispersely, would not sporulate, and mated prolifically. Two auxotrophic mutants, WY91 and WY92, were obtained from 10^4 colonies of WY42 screened by replica plating after ultraviolet irradiation.

WY91 showed normal respiration (R^+) and had an absolute requirement for arginine and a partial requirement for leucine. Petite (R^-) derivatives, 91-LP1 to LP9 ($\alpha\ Arg^-R^-$), were obtained by acriflavine treatment.[2]

WY92 required lysine and was also R^-, as are many of the survivors after ultraviolet irradiation.[3]

WY115 was obtained from 10^4 colonies of WY44 by similar methods. It responded to the thiazole component of vitamin B_1 and was R^-. An $aThz^-R^+$ segregant, WY110, was obtained from a hybrid of WY115 and WY42.

An actidione-resistant mutant of WY110, 110-Ac3 ($aThz^-Ac^rR^+$), was selected on complete medium[4] containing 10 mg. actidione per liter.

Ascus analysis of hybrids of WY92 and WY44 (H1, 2, 3) indicated that the former was a segregational petite[5] (i.e., non-cytoplasmic) and was therefore unsuitable for the projected experiments. 3:1 and 4:0 segregations for lysine were obtained. Non-mating, directly sporulating spore clones were recovered from these hybrids and from a hybrid of WY91 and 110-Ac3 (H4). Thirty asci from H4 were dissected, but only 26 spores from 18 asci were recovered.[6] The only tetrad completely recovered produced three prototrophic clones and one clone which required both arginine and thiazole.

X-ray survival tests on WY42 and a hybrid (WY91 × WY44) indicated that both of the parental stocks were already diploid. Presumably they were homozygous for mating type.

All the spores recovered from H4 showed some recombination of the parental markers, which are therefore not closely linked. H4 was actidione-resistant, though less so than 110-Ac3, and the segregants showed a range of levels of actidione resistance.

EXPERIMENTAL DESIGN AND PROCEDURE

According to Fowell's observations, many buds from mating pairs should have parental combinations of genes. In addition, because of the possibility of cytoplasmic mixing in a fusion figure, some buds from $R^- \times R^+$ mating pairs should receive R^+ cytoplasm with the gene combination of the R^- parent. Their mani-

festation of the R^+ phenotype would indicate extranuclear transmission of the normal respiratory factor. Conversely, extranuclear transmission of the suppressive factor[7] would be indicated by the isolation of R^- buds containing the other markers of the R^+ parent.

$R^- \times R^-$ and $R^+ \times R^+$ crosses were examined in control experiments.

In the experiments to be reported, mass-mating mixtures were incubated for 4–8 hours before the isolation of mating pairs was begun.[8] An earlier series of experiments, in which 72 pairs were isolated 11–24 hours after setting up the mating mixtures, had not given any evidence of cytoplasmic transfer.

Each mating pair was incubated until it had formed a progeny of 20–100 cells, which was then spread on a plate of complete medium. This was replica-plated to minimal[9] and complete media to detect auxotrophs among the progeny. Any auxotrophs were characterized for growth requirements by replica plating to a series of appropriately supplemented plates of minimal medium. Respiratory status was usually determined in tubes of acetate broth,[10] but in control experiments a plate of acetate medium was included in the series of replica plates.

Sometimes buds were removed from the isolated mating pairs and cultured separately for characterization of growth requirements and respiratory status.

Samples of auxotrophs from each plate were tested for mating type. Actidione resistance was tested in crosses involving 110-Ac3 or its R^- derivatives.

ORIGIN OF THE PARENTAL-TYPE BUDS FROM MATING PAIRS

The crosses involved WY91 or its R^- derivatives (α Arg^-) and WY110 or its actidione-resistant or petite derivatives ($aThz^-Ac^r$ or Ac^s). Auxotrophs were found in the progeny of 91 out of 530 mating pairs which did not have buds present at the time of isolation or were stripped of existing buds. Thirty-three of these pairs yielded both types of auxotrophic progeny, and 58 gave one or the other type. The remaining 439 pairs gave exclusively prototrophic (hybrid) progeny. Prototrophic progeny were also present on most of the plates containing auxotrophs.

In 90 of the 91 sets of auxotrophic progeny, no recombination of the parental markers was observed. The remaining set, produced by a pair from the mating WY91 $\times$ 110-Ac3, contained Thz^- progeny, which, unlike the parental cultures, would not mate with either WY42 or WY44 and could be induced to sporulate, although poorly. They also showed an intermediate level of actidione resistance which, together with the weak sporulation, suggests that these auxotrophs were aneuploid hybrids. At best, this is a dubious case of recombination.

Therefore, it can be concluded that the auxotrophs carry nuclei assorted from heterokaryons without intervening fusion, which would afford an opportunity for genic recombination. Thus any changes in respiratory status depend on extranuclear transmission or on mutation of nuclear or cytoplasmic determinants of respiration.

EXTRANUCLEAR TRANSMISSION

$R^- \times R^+$ matings.—The suppressiveness of three of the 91-LP series of petites was determined.[7] An R^+ histidine-requiring culture,[11] WY121, was used as the screening parent. 91-LP9 (αArg^-R^-) was found to be 76 per cent suppressive and was used in a series of crosses with WY110 ($aThz^-R^+$). The results of these and

all subsequent experiments are given in Table 1. One case of a change from αArg^-R^- to αArg^-R^+ was found among seven sets of progeny containing Arg^- auxotrophs. In the same experiments, three of eight pairs which yielded Thz^- progeny gave some $aThz^-R^-$.

TABLE 1

RESULTS OF EXPERIMENTS WITH MATING PAIRS ISOLATED AFTER 4–8 HOURS INCUBATION OF MATING MIXTURES

TYPE OF EXPERIMENT	NUMBER OF PAIRS ISOLATED	NUMBER OF PAIRS GIVING AUXOTROPHIC PROGENY			NUMBER OF PAIRS GIVING AUXOTROPHS OF CHANGED RESPIRATORY STATUS	
		Arg^-	Thz^-	Both Types	Parent α	Parent a
$R^- \times R^+$						
91-LP9* × WY110 ($\alpha Arg^-R^- \times a\ Thz^-R^+$)	71	4	5	3	1	3
91-LP3† or 5‡ × WY110 or 110-Ac3 ($\alpha Arg^-R^- \times a\ Thz^-(Ac^r)R^+$)	147	4	15	8	4	3
$R^- \times R^-$						
91-LP3 or 5 × 110-P3, 4, or 5 ($\alpha Arg^-R^- \times aThz^-R^-$)	268	7	8	11	0	0
$R^+ \times R^+$						
WY91 × WY110 or 110-Ac3 ($\alpha Arg^-R^+ \times aThz^-(Ac^r)R^+$)	135	7	12	14	0	0

* 76 per cent suppressive.
† 27 per cent suppressive.
‡ 4 per cent suppressive.

The next series of tests involved crosses of 91-LP3 (27 per cent suppressive) or 91-LP5 (4 per cent suppressive) with WY110 or 110-Ac3. Twelve pairs yielded Arg^- progeny, and 4 of these pairs gave some $\alpha\ Arg^-R^+$ types, while only 3 of 23 pairs which gave Thz^- progeny showed a change from R^+ to R^-.

Thus the number and direction of the changes observed showed the expected correlation with the suppressiveness of the R^- parent.

$R^- \times R^-$ Control Matings.—The occurrence of the mutation from R^- to R^+ has never been definitely established, and the $R^- \times R^-$ crosses so far reported have given exclusively R^- progeny, unless one of the petites was segregational. However, a series of $\alpha\ Arg^-R^- \times aThz^-R^-$ crosses (91-LP3 or 5 × 110-P3, 4- or 5-acriflavine R^- cultures from WY110) was made as a control for the changes from $\alpha\ Arg^-R^-$ to $\alpha\ Arg^-R^+$ which were observed in the $R^- \times R^+$ crosses.

The progeny plates contained exclusively small-colony types, and these were all found to be R^- on examination of the acetate replica plates. Seven pairs gave $\alpha\ Agr^-R^-$ progeny, 8 gave $aThz^-R^-$, and 11 gave a mixture of these two types of auxotrophs. Thus 18 pairs gave $\alpha\ Arg^-R^-$, and none gave $\alpha\ Arg^-R^+$, in comparison with the test series, where 5 of 19 pairs which yielded Arg^- progeny gave some $\alpha\ Arg^-R^+$.

$R^+ \times R^+$ Control Matings.—Mutation from R^+ to R^- is quite common during vegetative growth. Ephrussi *et al.*[12] calculated that the mutation rate is 2×10^{-3} per cell generation. $R^+ \times R^+$ crosses were carried out to determine whether the R^- parent (in $R^- \times R^+$ matings) had contributed to the observed frequency of changes from R^+ to R^- in thiazole auxotrophs.

Auxotrophic progeny were obtained from 33 of 135 pairs isolated from matings of WY91 and WY110 or 110-Ac3. All these were R^+. One mating pair, which

gave exclusively prototrophic progeny, contained 3 R^- among 100 R^+ colonies. A bud removed from this pair also gave rise to an R^- clone.

Seven pairs gave α Arg^-R^+ progeny, 12 gave a Thz^-R^+, and 14 gave a mixture of the two types of auxotrophs. With the exception noted above, all the prototrophic progeny were also R^+. Thus 26 pairs gave $aThz^-R^+$ progeny, and none gave $aThz^-R^-$, in comparison with the test series, where 6 of 31 pairs which yielded $aThz^-$ progeny gave some $aThz^-R^-$. Exact treatment[13] of the 2 × 2 tables, 18/0:14/5 for Arg^- progeny and 26/0:25/6 for Thz^- progeny, gives $p = 0.03$ and 0.02 that the observed changes in respiration are the same for $R^- \times R^+$ versus $R^- \times R^-$ and for $R^- \times R^+$ versus $R^+ \times R^+$ crosses, respectively.

If mutation is independent of the other parent in the mating, it cannot account for the observed ahanges. The changes from R^- to R^+ and from R^+ to R^- are therefore probably caused by cytoplasmic transmission of the factors responsible for normal respiration and the suppression of normal respiration in accord with the conclusions of Ephrussi *et al.*[2, 7]

The interested reader is referred to Wright[14] for technical details and a discussion of remaining problems concerning these factors.

SUMMARY

Fowell's interpretation that parental-type buds from mating pairs in a strain of *S. cerevisiae* var. *ellipsoideus* arise from transient heterokaryons has been confirmed. In a series of experiments with doubly and triply marked stocks, carrying markers which were not closely linked, no unequivocal case of recombination was found.

Progeny of isolated heterokaryons include clones of the parental genotype which show a change in respiratory phenotype. The latter therefore appears to be subject to extranuclear transmission, in accord with the conclusion of Ephrussi *et al.*

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[1] R. R. Fowell, *J. Inst. Brewing*, **57**, 180–195, 1951.

[2] B. Ephrussi, *Nucleo-cytoplasmic Relations in Micro-organisms* (London: Oxford University Press, 1953).

[3] C. Raut, *J. Cellular Comp. Physiol.*, **44**, 463–475, 1954.

[4] Glucose, 10 gm.; tryptone (Difco), 5 gm.; yeast extract (Difco), 1 gm.; KH_2PO_4, 2 gm.; K_2HPO_4, 0.8 gm.; zinc (as sulfate), 400 μg.; iron (as ferrous ammonium sulfate), 150 μg.; copper (as sulfate), 25 μg.; water, 1 liter.

[5] S. Y. Chen, B. Ephrussi, and H. Hottinguer, *Heredity*, **4**, 337–51, 1950.

[6] Four-spored asci were obtained from slants of Fowell's sporulation medium (*Nature*, **170**, 573, 1952). Asci were dissected in a moist chamber, with the aid of a DeFonbrune micromanipulator. Spore viability was low; only 27 per cent of all spores isolated formed visible colonies. Many others formed small clones (4–50 cells) which did not develop further.

In order to decrease the possibility of spore damage during ascus breakage, a wall-softening enzyme was obtained by growing *Aerobacillus polymyxa* (strain C7, kindly supplied by Dr. Elizabeth McCoy, Bacteriology Department, University of Wisconsin) for 4 days at 25° C. in the presence of yeast asci suspended in nutrient broth containing 1 per cent NaCl. After centrifugation, solid ammonium sulfate, to give *ca.* three-fourths saturation, was added to 10 ml. of the supernate. The precipitate was dissolved in 1 ml. of sterile distilled water. After incubation of fresh asci for

3 hours in this preparation, many free spores were present, and intact asci broke after a minimum of manipulation.

[7] B. Ephrussi, H. Hottinguer, and H. Roman, these PROCEEDINGS, **41**, 1065–1071, 1955.

[8] The oil-chamber technique of P. DeFonbrune, *La Technique de micromanipulation* (Paris: Masson & Cie, 1949), was used.

[9] The synthetic medium of B. H. Olson and M. J. Johnson (*J. Bacteriol.*, **69**, 159–162, 1949) was used. Asparagine was omitted, and biotin and calcium pantothenate were the only vitamins added. This was supplemented as required with amino acids (10 mg/l) or 4-methyl-5-β-hydroxyethyl thiazole (0.1 mg/l).

[10] M. Ogur, G. Lindegren, and C. C. Lindegren, *J. Bacteriol.*, **68**, 391–392, 1954. This medium was modified for replica plating by omitting the indicator, reducing the glucose to 0.025 per cent, and adding agar.

[11] Kindly supplied by Dr. S. Spiegelman as 33-E-26.

[12] B. Ephrussi, P. L'Héritier, and H. Hottinguer, *Ann. Inst. Pasteur*, **77**, 64–83, 1949.

[13] R. A. Fisher, *Statistical Methods for Research Workers* (Edinburgh: Oliver & Boyd, 1944).

[14] R. E. Wright, *Extranuclear Inheritance in Yeast Heterokaryons* (doctoral dissertation, University of Wisconsin, 1957) (University Microfilms, 313 N. First St., Ann Arbor, Michigan).